AEC-NASA TECH BRIEF

Tritiated Alumina Serves as Reagent for Self-Labeling Analysis

The problem:

To develop a method of exchange-labeling of specific compounds in low concentrations prior to chromatographic analysis. The requirements for the achievement of such an analytical method are: (1) the preparation of a uniform, reproducible absorbent of known specific activity and exchange capability; (2) the establishment of the linearity of exchange reaction with the sample size; (3) knowledge of the degree to which all exchangeable positions in the molecule participate in the labeling process; and (4) the effective resolution of one class member from another by the chromatographic column.

The solution:

Tritiated alumina, prepared by exchange of the surface hydroxyl groups with tritiated water, is a suitable reagent. When put in a chromatographic column, it effects the labeling of compounds passed over it. Columns of this material provide the ability to detect and measure submicrogram quantities of material. The use of radioactive hydrogen permits the gleaning of information through radioactive measurements that previously required mass spectrometer measurements.

How it's done:

One hundred grams of alumina were heated at 1-mm pressure for 2 hours, cooled in vacuo, and deactivated with 3 ml of tritiated water. The rehydrated alumina was permitted to equilibrate and again was subjected to heat at reduced pressure. This time, and all subsequent times, the water obtained during the dehydration process was collected; the volume and specific activity of this collected water were measured. Usually four to six cycles were sufficient to achieve complete equilibration of the alumina surface with the tritiated water used in rehydration.

The specific activity of the water removed by high temperature dehydration is only an approximate indication of the specific activity of the alumina surface, particularly when the number of cycles is small. Therefore, a small portion of the alumina prepared in each cycle was set aside to be used for a test chromatographic column. Coprostanone was used as the test sample, and the total activity per mg was measured by liquid scintillation counting after complete elution from the column. This procedure provides a means of standardizing the specific activity by comparison to a known reference.

In this process, the previously unlabeled constituents of a suitable class became radioactive during their passage through, and separation by, the chromatographic column to a degree that reflects both their concentration and those molecular features susceptible to labeling. To determine whether a proportionate response could be obtained over a wide range of sample sizes, the reference sample (a ketosteroid) was run at levels from 10 mg to 3μg, and the recovery of activity was determined. The results indicated that a direct linear relationship exists between the weight of sample applied and the amount of radioactivity appearing in the chromatographic peak. The results also indicated that the tritiated alumina may be diluted with unlabeled alumina of the same adsorbent activity to obtain sample labeling activities that are directly proportional to the dilution.

There are two alternative capabilities of self-labeling analysis: (1) knowing the constituents of a mixture, their individual concentrations can be determined radiochemically; or (2) knowing the amounts of each, the degree of substitution on the alpha carbons can be ascertained by determining the specific activity as a multiple of the adsorbent activity.

(continued overleaf)

Notes:

1. A mixture of ketosteroids was tested using 30% benzene/pentane. The area under each peak was totaled and the recovery of the samples calculated from the relationship: Sample weight = (c.p.m. in peak) × (molecular weight/(aliquot) × (efficiency) × (surface specific activity) × (theoretical number of enolic H) × (2.22×10^9). The results showed good recovery.
2. In addition to ketosteroids, this method is applicable to other detonic compounds which can be chromatographic on alumina.
3. It is not necessary to know the specific activity of the HTO used in the preparation of the reagent or the number of times the absorbent has been equilibrated.
4. The detection limits of columns of tritiated alumina appear to extend to submicrogram quantities.
5. Additional details are contained in *Analytical Chemistry*, vol. 38, March 1966, p. 480–484.
6. Inquiries concerning this innovation may be directed to:

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Patent status:

Inquiries about obtaining rights for commercial use of this innovation may be made to:

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